abcam

Product datasheet

Anti-Rb antibody [E182] - BSA and Azide free ab239820

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-Rb antibody [E182] - BSA and Azide free
Description	Rabbit monoclonal [E182] to Rb - BSA and Azide free
Host species	Rabbit
Specificity	The antibody detects pan Rb protein.
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, ICC/IF, WB **Unsuitable for:** IHC
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Hek293, and Jurkat cell lysates; ICC: A431 cells; Flow Cyt (intra): Jurkat cells.
General notes	ab239820 is the carrier-free version of **ab32513**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E182
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab239820 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 106 kDa).

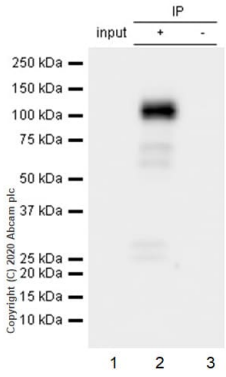
Application notes Is unsuitable for IHC.

Target

Function Key regulator of entry into cell division that acts as a tumor suppressor. Promotes G0-G1 transition when phosphorylated by CDK3/cyclin-C. Acts as a transcription repressor of E2F1 target genes. The underphosphorylated, active form of RB1 interacts with E2F1 and represses its transcription activity, leading to cell cycle arrest. Directly involved in heterochromatin formation by maintaining overall chromatin structure and, in particular, that of constitutive heterochromatin by stabilizing histone methylation. Recruits and targets histone methyltransferases SUV39H1, KMT5B and KMT5C, leading to epigenetic transcriptional repression. Controls histone H4 'Lys-20' trimethylation. Inhibits the intrinsic kinase activity of TAF1. Mediates transcriptional repression by SMARCA4/BRG1 by recruiting a histone deacetylase (HDAC) complex to the c-FOS promoter. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC1 repressor complex. Upon calcium influx, RB1 is

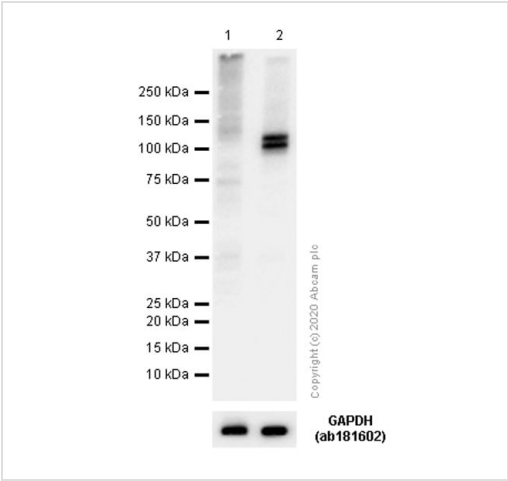
	dephosphorylated by calcineurin, which leads to release of the repressor complex (By similarity). In case of viral infections, interactions with SV40 large T antigen, HPV E7 protein or adenovirus E1A protein induce the disassembly of RB1-E2F1 complex thereby disrupting RB1's activity.
Tissue specificity	Expressed in the retina.
Involvement in disease	Childhood cancer retinoblastoma Bladder cancer Osteogenic sarcoma
Sequence similarities	Belongs to the retinoblastoma protein (RB) family.
Domain	The Pocket domain binds to the threonine-phosphorylated domain C, thereby preventing interaction with heterodimeric E2F/DP transcription factor complexes.
Post-translational modifications	Phosphorylated by CDK6 and CDK4, and subsequently by CDK2 at Ser-567 in G1, thereby releasing E2F1 which is then able to activate cell growth. Dephosphorylated at the late M phase. SV40 large T antigen, HPV E7 and adenovirus E1A bind to the underphosphorylated, active form of pRb. Phosphorylation at Thr-821 and Thr-826 promotes interaction between the C-terminal domain C and the Pocket domain, and thereby inhibits interactions with heterodimeric E2F/DP transcription factor complexes. Dephosphorylated at Ser-795 by calcineruin upon calcium stimulation. CDK3/cyclin-C-mediated phosphorylation at Ser-807 and Ser-811 is required for G0-G1 transition. Phosphorylated by CDK1 and CDK2 upon TGFB1-mediated apoptosis. N-terminus is methylated by METTL11A/NTM1 (By similarity). Monomethylation at Lys-810 by SMYD2 enhances phosphorylation at Ser-807 and Ser-811, and promotes cell cycle progression. Monomethylation at Lys-860 by SMYD2 promotes interaction with L3MBTL1. Acetylation at Lys-873 and Lys-874 regulates subcellular localization, at least during keratinocytes differentiation.
Cellular localization	Nucleus.

Images



Immunoprecipitation - Anti-Rb antibody [E182] - BSA and Azide free (ab239820)

This data was developed using **ab32513**, the same antibody clone in a different buffer formulation.
Purified **ab32513** at 1/40 dilution (2µg) immunoprecipitating Rb in Jurkat whole cell lysate.
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10µg
Lane 2 (+): **ab32513** + Jurkat whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32513** in Jurkat whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 120 kDa



Western blot - Anti-Rb antibody [E182] - BSA and Azide free (ab239820)

All lanes : Anti-Rb antibody [E182] (**ab32513**) at 1/1000 dilution (Purified)

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) prepared in RIPA lysis method whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) prepared in 1% SDS Hot lysis method whole cell lysate

Lysates/proteins at 15 µg per lane.

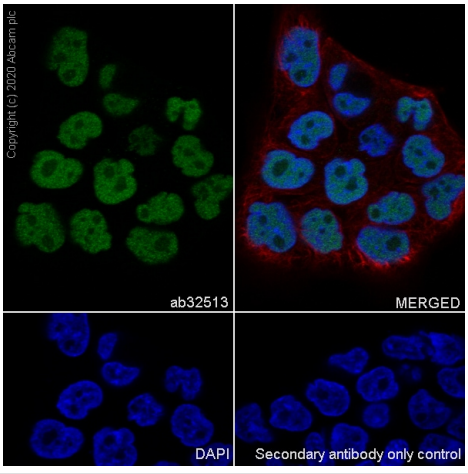
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 106 kDa
Observed band size: 106-120 kDa

This data was developed using **ab32513**, the same antibody clone in a different buffer formulation.

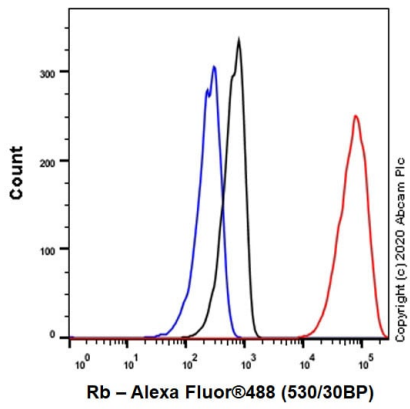
1% SDS Hot lysis method is preferred for this antibody.

Blocking Buffer and concentration: 5% NFDM/TBST



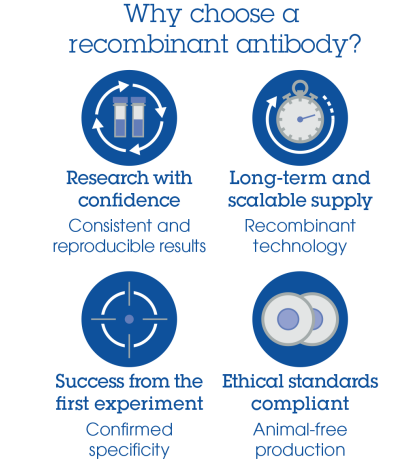
Immunocytochemistry/ Immunofluorescence - Anti-Rb antibody [E182] - BSA and Azide free (ab239820)

This data was developed using **ab32513**, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling Rb with Purified ab239820 at 1:50 dilution (10 ?g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Rb antibody [E182] - BSA and Azide free (ab239820)

This data was developed using **ab32513**, the same antibody clone in a different buffer formulation.Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Rb with Purified ab239820 at 1/70 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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